Drosophila development: **Scalloped and Vestigial take wing**

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The proteins Scalloped and Vestigial are known from genetic studies to play a part in *Drosophila* wing development. Recent results show how they interact with each other, and in combination with other transcription factors, to confer specific patterns of expression within the wing.

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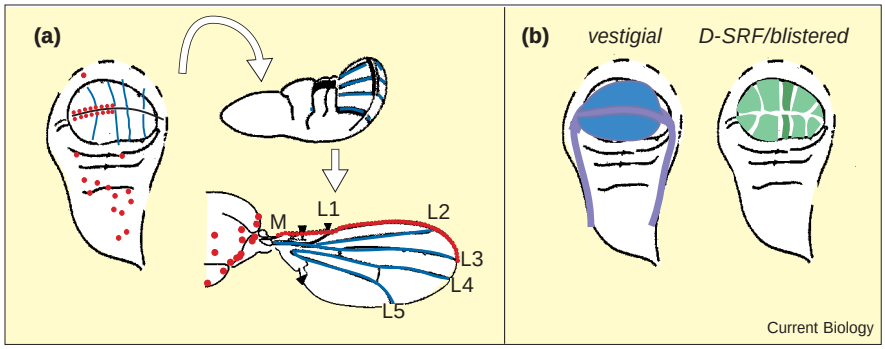
What makes a wing different from a leg or an eye? Any of us could list a variety of distinctions, but the mechanisms that confer these characteristics during development are less clear. In *Drosophila*, the progenitors of the adult structures are the so-called 'imaginal discs', epithelial discs which are set aside in the embryo, then proliferate and differentiate during larval and pupal development (Figure 1). Several key pathways involved in coordinating the growth and patterning of these imaginal discs have been identified. For example, the signalling molecules Hedgehog, Wingless and Decapentaplegic are essential at several stages of wing, leg and eye development, along with cell–cell communication mediated by the cell-surface protein Notch. What, then, confers the distinct responses to these signalling pathways in the individual appendages? Three recent papers [1–3] demonstrate the role of molecular interactions between the Scalloped (Sd) and Vestigial (Vg) proteins in promoting wing development, and indicate that a Sd–Vg complex acts in combination with transducers of the signalling pathways to promote transcription of genes required for wing morphogenesis [1].

The involvement of *vestigial* (*vg*) in *Drosophila* wing development is not of itself a new story: the reduced wing of *vg* mutants was first described by Thomas Hunt Morgan in 1910 [4], and is familiar to many school-children who use the *vg* mutant in their biology lessons. Ectopic *vg* expression is sufficient to convert cells in the eye, antenna and leg discs to wing-like fates [5]. In this respect *vg* appears analogous to *Pax6/eyeless*, the master switch or 'selector gene' for eye development [6]. The concept of a selector gene, as proposed by Garcia-Bellido [7], is of a switch that assigns a cell and its progeny to one developmental pathway. In many respects, *eyeless* fits the bill; its expression is unique to the eye, it is sufficient to switch other cells to an eye fate, and it encodes a DNA-binding protein that controls expression of genes needed for eye morphogenesis [6]. Although *vg* shares many of these properties with respect to wing development, the protein it encodes has a novel structure and does not contain a DNA-binding motif [8], so its mode of action has been a mystery.

The *scalloped* (*sd*) gene is also essential for wing development [4]. It differs from *vg*, however, in that its expression is much more widespread, being detected in other imaginal discs and the nervous system, and its misexpression results in a loss of wing tissues, rather than promoting novel wing development in other discs [1,3,9]. So *sd* does not have the characteristics of a wing-specific switch. Nevertheless, *sd* does encode a DNA-binding protein: specifically, Sd is a member of the TEA/ATTS class of transcription factors, homologous to the human protein 'transcription enhancer factor 1' (TEF-1), which binds to the simian virus 40 (SV40) enhancer [9–11].

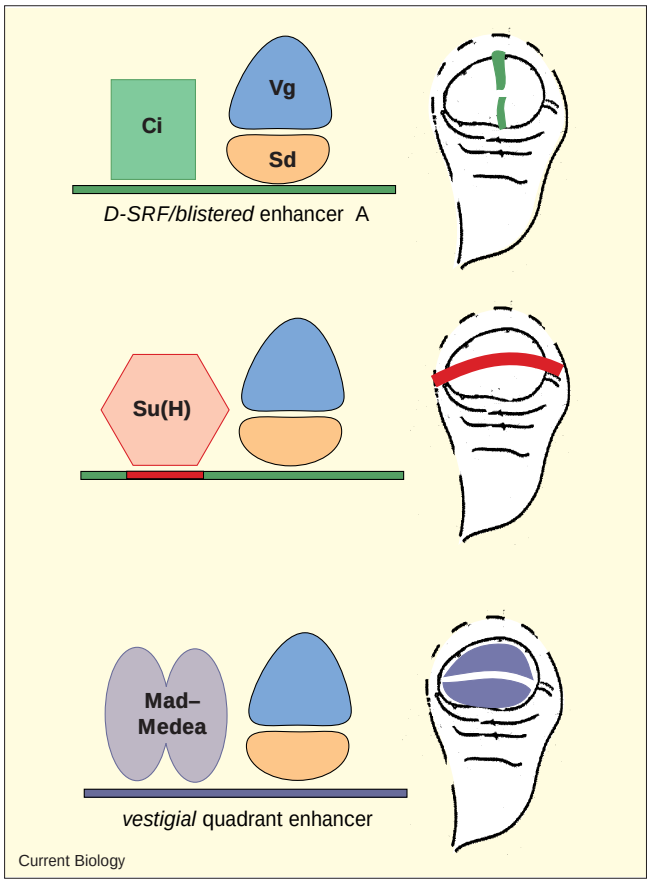
It now emerges that Sd is required for Vg to exert its effects. Thus, misexpression of Vg results in ectopic wing tissue only in places where Sd is also present [1,2]. This

Figure 1



Wing development in *Drosophila*. **(a)** Three stages are shown: late larval imaginal disc; everting pupal disc (rotated 90° with respect to the larval disc); and adult wing, with positions of sensory organs (red) and wing veins (blue) indicated. **(b)** Expression patterns of *vestigial* (purple) and *D-SRF/blistered* (green) in the wing disc; the darker shading delimits the expression from the specific enhancers depicted in Figure 2.

Figure 2



A model for combinatorial regulation of wing-specific enhancers by Sd–Vg and transcription factors at the ends of signalling pathways (see [3] and text for details).

correlates with the activation in these ectopic locations of 'wing' target genes, such as *D-SRF/blistered* (which is needed for the correct morphology of the intervein cells in the wing, see Figure 1) [1,3]. Conversely, when *sd* is removed, expression of *D-SRF/blistered* in the wing is abolished. But *sd* and *vg* are normally co-expressed in the wing and their expression is mutually dependent [12], so it was important to test whether *sd* is still required for *vg* function when this interdependence is circumvented by expressing Vg under heterologous regulation. Even under such conditions, expression of *D-SRF/blistered* and other target genes was abolished in *sd* mutant cells [1,3]. These observations suggest that Sd functions in parallel with Vg to coordinate the expression of wing genes such as *D-SRF/blistered*.

The parallel effects of Sd and Vg are explained by the fact that the two proteins interact directly, as shown by recent *in vitro* and yeast two-hybrid experiments [1–3]. In cell culture assays, Vg acts synergistically with Sd to stimulate expression from *D-SRF/blistered* and *vg* enhancers [1]. Deletion of the Sd-interaction domain from Vg renders it unable to activate wing target genes *in vivo* or to promote wing development [3]. Thus the two proteins together form a transcriptional activator, in which Sd provides the DNA-binding component and Vg is thought to provide the transcriptional activation domain, although this has not been formally proven. Consistent with this view, Sd binding sites are present in the wing-specific enhancers of target genes such as *D-SRF/blistered*, and wing expression is abolished when these sites are mutated [1,13]. The same enhancers also contain target sites for the effectors of specific signalling pathways — such as Cubitus interruptus (Ci), the transcription factor at the end of the Hedgehog signalling pathway, in the case of *D-SRF/blistered* — and it is proposed that these factors act in combination with Sd–Vg [1]. The idea is that the precise combination of signalling effectors and Sd–Vg that impinges on a particular target gene determines its specific pattern of expression.

The wing transcriptional enhancers are thus elegant examples of combinatorial gene regulation, with the Sd–Vg complex being the component that promotes wing-specific expression (Figure 2). One intriguing aspect of this model is that the specificity in the Sd–Vg complex does not reside in the DNA-binding component Sd, but in the restricted expression of Vg. If Sd interacts with alternative coactivators in other discs/tissues, as seems likely from the variety of phenotypes detected in Sd mutants [9], there must be a mechanism for targeting different Sd–coactivator complexes to specific enhancers.

One possibility is that the sequence-specificity of DNA recognition by Sd is augmented by the coactivator; this could be achieved, for example, if Vg itself contacts some bases or if it alters the stability of Sd–DNA interactions. There are many examples of cofactors that confer added DNA-binding specificity on a complex by contacting the DNA, as in the interactions of SAP1 and ELK-1 with serum response factor [14]. Furthermore, in the case of some heterodimers between Hox proteins and their Pbx–Extradenticle cofactors, the interaction induces a change in the binding affinity/specificity of the Hox protein [15].

Clearly, the Sd protein can bind to sites in the relevant enhancers in the absence of Vg [1], but so far there has been no comparison with the binding of the Sd–Vg complex to see whether the affinity is altered by the presence of the coactivator. If Vg does not affect the DNA-binding properties of Sd, it would suggest the alternative that the topology of the Sd–Vg complex is critical for conferring specificity: that is, that a combination of Sd with a different coactivator would not be able to cooperate effectively with the other transcription factors bound to the wing enhancers. Given the apparently modular nature of enhancers, this seems a less likely explanation, but resolution of the issue must await the identification of additional Sd coactivators.

A further question to be resolved is whether there are indeed other partners for Sd and, if so, whether they resemble Vg. The *sd* mutant phenotypes include defects in sensory organ development and *sd* transcripts can be detected in both peripheral and central nervous system [9]. Perhaps Sd has a different partner in these tissues, but so far no proteins similar to Vg have been identified in flies or vertebrates, although there is evidence that tissue-specific factors associate with the human TEF-1. Expression of *vg* itself is also not unique to the wing; although Vg cannot be detected in other imaginal discs, *vg* is expressed in a subset of somatic muscles [16]. There is no evidence for *sd* expression in these cells, so presumably Vg interacts with another DNA-binding protein that will direct the complex to a distinct set of target genes. This emphasises that it is the Sd–Vg combination that directs wing development, rather than Vg per se.

A comparison between different wing-specific enhancers gives rise to a simple model of combinatorial regulation whereby Sd–Vg acts in combination with different signalling inputs (Figure 2). Thus, an enhancer combining Sd–Vg and Ci binding sites gives a stripe of expression adjacent to the posterior compartment where Hedgehog is expressed [6]. When a different site is substituted for that of Ci, the pattern of expression is changed: thus in the example illustrated in Figure 2, the new binding site is for Suppressor of Hairless (Su(H)) and the expression pattern is changed to a dorsal–ventral stripe, reflecting the activity of the Notch pathway that operates through this DNA-binding protein. Likewise, an enhancer containing a binding site for Mad–Medea, the effector of the Decapentaplegic pathway, gives rise to broader expression throughout the wing field [17].

A model of this kind, involving the combined action of signalling pathways with tissue-specific elements, has also been proposed from studies of Dpp target genes in the embryo, where the transcription factor Mad–Medea combines with complexes such as Labial–Extradenticle in the endoderm or Tinman in the mesoderm [18,19]. It is clear, however, that more factors will be involved; for example the enhancer that responds to Mad–Medea and Sd–Vg is not active throughout the wing field, there is a domain where it is silent. Furthermore, many of the wing enhancers are also targets for Ultrabithorax [20], which is required to repress wing-specific genes in the haltere. As the relationships between these different components are mapped onto the DNA of enhancers for genes such as *D-SRF/blistered*, we begin to get a picture of the molecular code that specifies morphologically distinct structures, such as wings, eyes and legs.

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